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Seasonal variation in the nitrogen mineralization and mineral nitrogen accumulation in two temperate forest soils

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With 4 figures

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1. Introduction

Nitrogen, one of the major plant nutrients, has its principal source in the soil organic residues. These substances, through the processes of microbial degradation, supply among other elements, the nitrogen which is utilized for protein synthesis (with the exception of a few nitrogen fixing microorganisms). Nature of the transformations of organic N in the soil, the role of the microorganisms and influence of local physical and chemical factors have been dealt with in numerous analytical studies under controlled conditions in the laboratory. Bremner (1965) concluded that incubation methods are the most reliable means of predicting nitrogen needs. The amount of NO_3^--N accumulated over an extended incubation period represents net N mineralization (Juma *et al.*, 1984). This has been found to be the most useful method for determining the effect of environmental and soil variables such as temperature, moisture, texture, *p*H, organic matter and its quality (Stanford & Epstein, 1974; Herlihy, 1979; Campbell *et al.*, 1981; Sollins *et al.*, 1984). Ecosystem studies, intensive harvesting techniques, potential use of wood for energy, and concern about nitrous oxides in the atmosphere have focused interest on the transformations of nitrogen in forest soils (Borman *et al.*, 1977; Freney *et al.*, 1978; Vitousek *et al.*, 1979; Keeney, 1980).

On the other hand, the *in situ* study of the processes that govern the gains and losses of soil N resulting from transformation and reorganisation/immobilization, and the exchange with the external medium by water circulation, absorption by roots, emission of nitrogen gases and biological fixation of nitrogen during different seasons provide the possibility to establish N balance in a given ecosystem (Lemée, 1967; Billes *et al.*, 1971 a, b).

The objectives of this paper were to study the influence of seasonal variations on the mineralization of soil organic N, and mineral N contents in an acid mull and an anmoor. These soils have originated from the same parent material but have different properties owing to their position within a topographical sequence.

2. Materials and methods

2.0. General note

The experimental sites, the climate, the vegetation, the total soil microbial count and the seasonal variation of the rate of organic C and N have been described elsewhere (Rashid & Schaefer, 1984). In all experiments composite samples were collected from a depth of 0—5 cm and passed through a 2 mm sieve. Roots and other recognisable debris were removed.

2.1. Experiment I

— The seasonal dynamics of mineral N *in situ:*

Samples were collected every 10 to 15 d between December 1974 and November 1976 with an interruption of two months (August and September, 1975). NH_4^+ and NO_3^--N contents were determined by Richardson's modification of Olsen's method as described by Piper (1950).

2. 2. Experiment II

— The seasonal dynamics of N mineralization *in situ* at a moisture content adjusted to 85 % of field capacity:

Samples were collected at the beginning of each season during 1975 and 1976. In acid mull (texture sandy loam; organic matter 4 %; *p*H 4.5) humidity was adjusted by adding distilled water wherever necessary; in the anmoor (texture loamy sand; organic matter 16 %; *p*H 7.5), where the moisture level varied between 250—400 % round the year (dry-basis, measured twice a week at 0—5 cm depth during 1975 and 1976), this was done by partial air-drying. The initial soil NH_4^+ and NO_3^--N were determined after moisture adjustment. The soils were then put into metallic boxes of 250 ml volume (7.5 cm diameter), pressed gently and then covered with perforated lids (hole diameter 1 mm) to allow the diffusion of gases (Lemee, 1967; Billes *et al.*, 1971). The boxes were placed upside down at 2—3 cm below the soil surface at the same experimental site (acid mull zone). In total there were 9 boxes for each soil. Three boxes for each soil were withdrawn every 3 weeks up to 9 weeks experimental period. NH_4^+ and NO_3^--N were determined by the method mentioned earlier. Loss of moisture through evaporation varied between 4—9 % (depending on season) during the experimental period.

2.3. Experiment III

— Seasonal dynamics of N mineralization *in situ* at a moisture content adjusted to 85 % of field capacity:

(a) The sampling dates and method of moisture adjustment were the same as in Experiment II. In this case 50 g portions of each soil were placed into 500 ml Erlenmeyer flasks, plugged loosely with cotton to permit gaseous diffusion and incubated at 28 °C. The loss of moisture was readjusted every 24 h. Duplicate samples were withdrawn for the determination of NH_4^- and NO_3^--N after 14, 28 and 42 d of incubation.

(b) In this experiment the flasks were tightly closed with rubber bungs and incubated at 28 °C for 3 weeks. Forced ventilation was applied twice or thrice a week to insure aeration (Rashid, 1977). Loss of moisture, if any, was readjusted periodically. Mineral N was determined on duplicate samples for each soil.

The rate of mineralization (Rm) was calculated by dividing the total mineral N (NH^+ and NO_3^--N) by the total soil N and multiplying by 100.

3. Results and discussion

Figure 1 presents the mineralization of soil N content (NH^+_4 and NO_3^--N) at 0—5 cm depth in the acid mull and anmoor, determined at different times as mentioned. In the mull, the rate of evolution of mineral N showed a sharp bimodal curve where the maxima fluctuated from one year to another. After an absolute minimum in the winter of 1975 and at the end of autumn of 1976, we observed at first a relatively brief phase of accumulation, then a relative minimum in the spring (May—June, 1975 and March-April 1976). This was followed by a second phase in the course of which the soil was again enriched in mineral N. In 1976 the sequential determination of mineral N went on uninterrupted. Here, however, the second phase of accumulation started in May, reaching its peak in September. The maintenance of an important mineral N reserve in mull during a long period might be linked with the particular meteorological conditions of 1976; the drought might have restricted the leaching losses and reduced the rate of absorption by roots (Rashid & Schaefer, 1985).

In the anmoor the mineral N content was much more variable. It was at a minimum in winter and at a maximum in spring (May) during 1975 whereas in 1976 the mineral N content evolved more or less in the same manner as in mull with two phases of accumulation. The first, a brief one, occurred in January and the second, a long one, extended from May to November with periodical regressions.

In both soils NH^+_4 accounted for a larger share of mineral N, this being more pronounced in the anmoor than in the mull. This might be due to the hydromorphic nature of the former soil initiating leaching out and absorption of NO_3^- by plants throughout the year. In the anmoor NH_4^+ might be formed partly through deamination of organic matter (Bremner & Shaw, 1958; Patrick & Wyatt, 1964; Macrae *et al.*, 1968), whereas in the mull leaching losses could take place only periodically. In addition to biotical immobilization, absorption by roots and leaching losses, NO_3^- is prone to denitrification (Broadbent & Clark, 1965; Robertson, 1984).

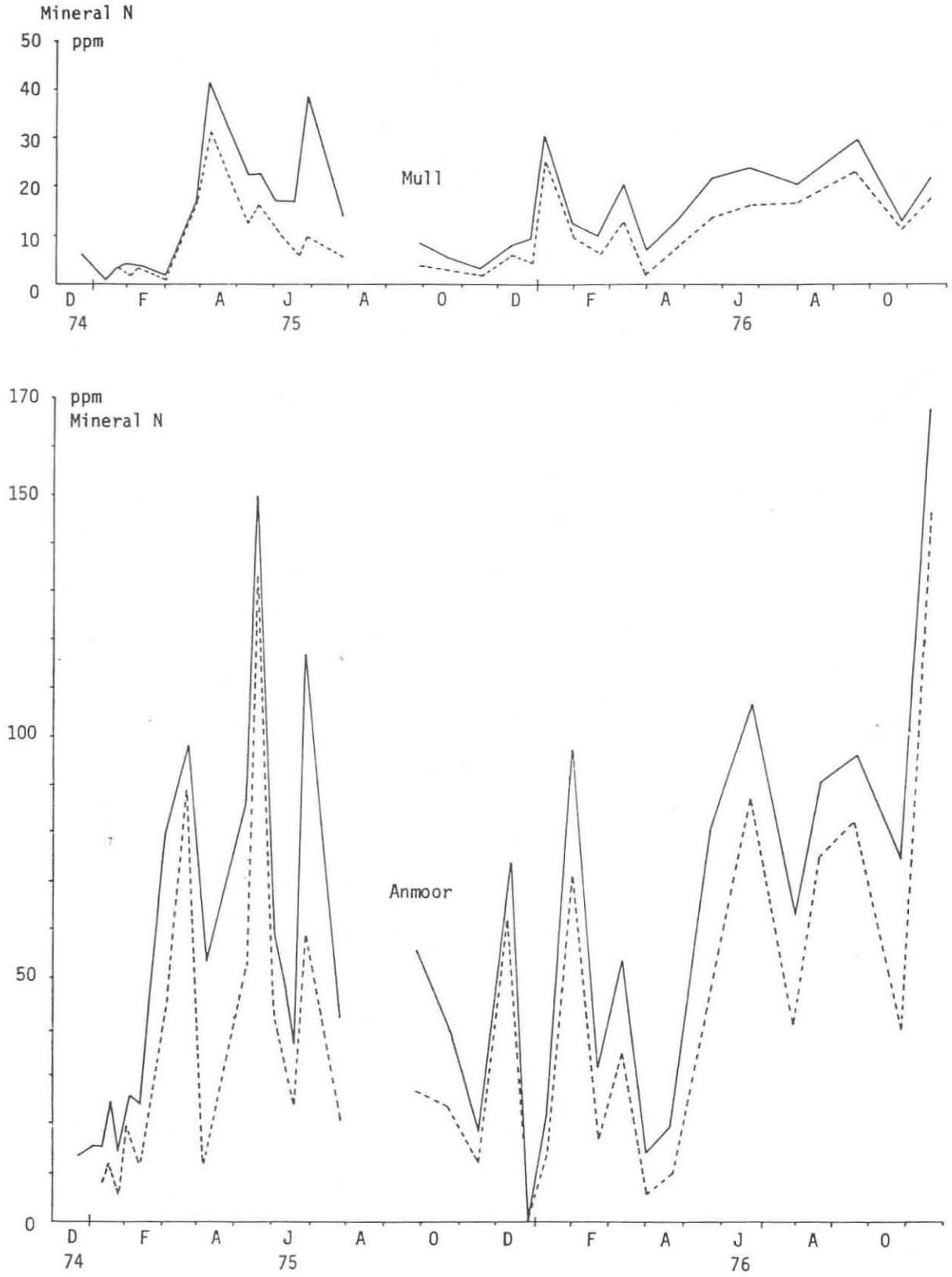

Fig. 1. Seasonal variation of *in situ* nitrogen mineralization.
$NH_4^+ + NO_3^-$-N ——— NH_4^+-N -------

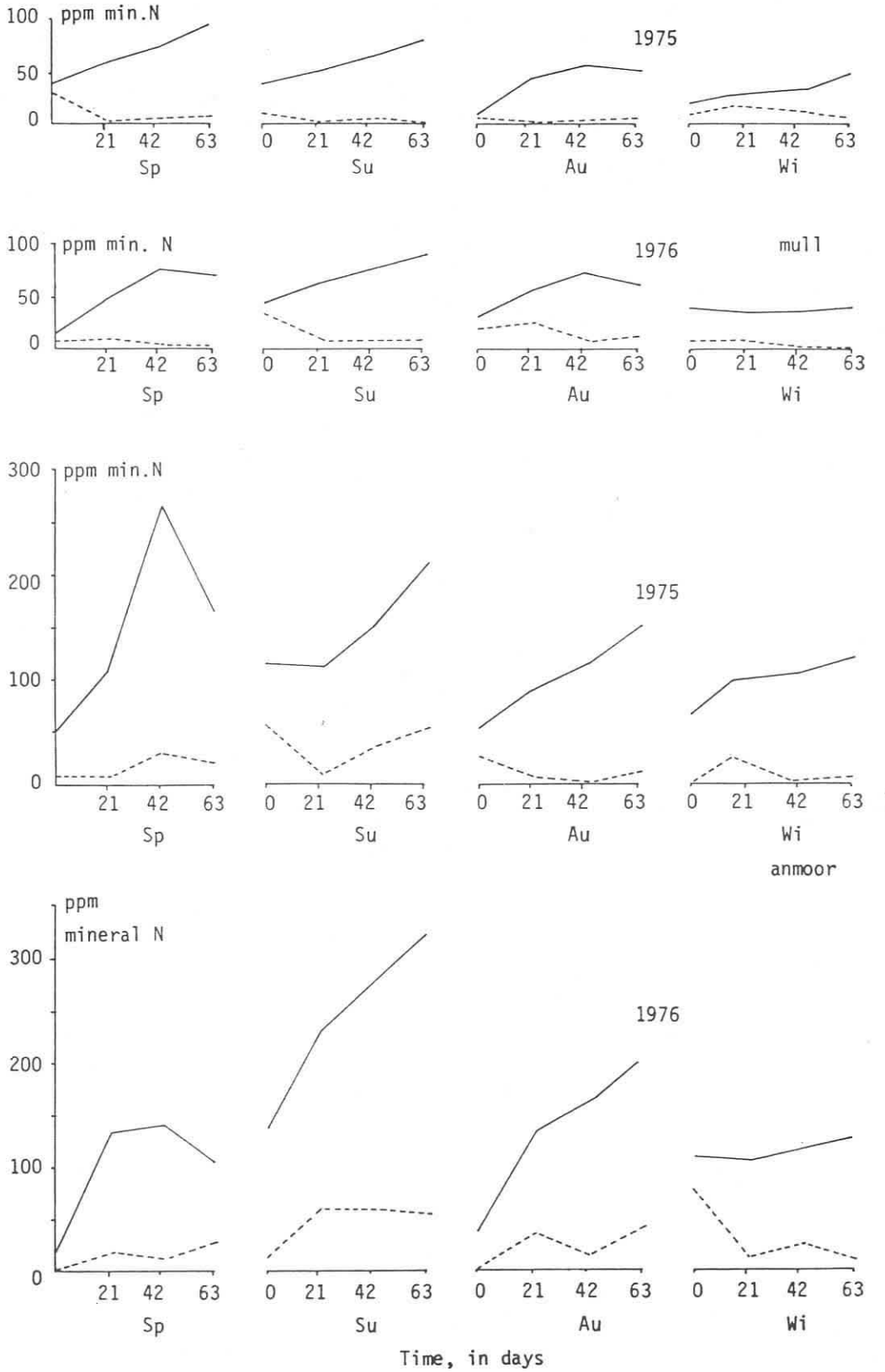

Fig. 2. Seasonal variation of nitrogen mineralization of soil adjusted to 85 % of field capacity, placed *in situ* in boxes.
$NH_4^+ + NO_3^-$-N ——— NH_4^+-N -------

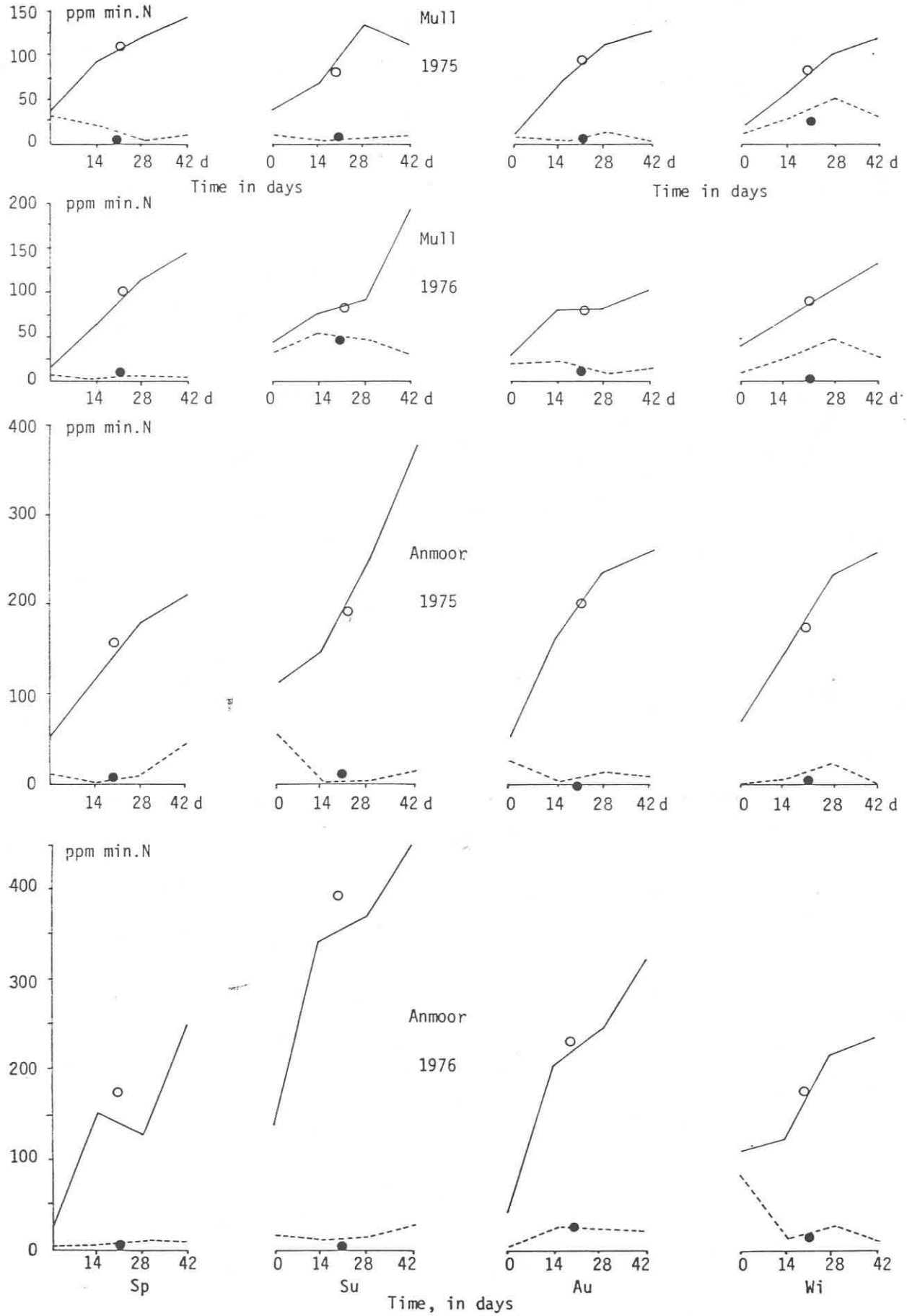

Fig. 3. Seasonal variation of *in vitro* nitrogen mineralization of soil adjusted to 80 % of field capacity and incubated at 28 °C.

$NH_4^+ + NO_3^-$-N ○——— NH_4^+-N ●------- forced aeration.

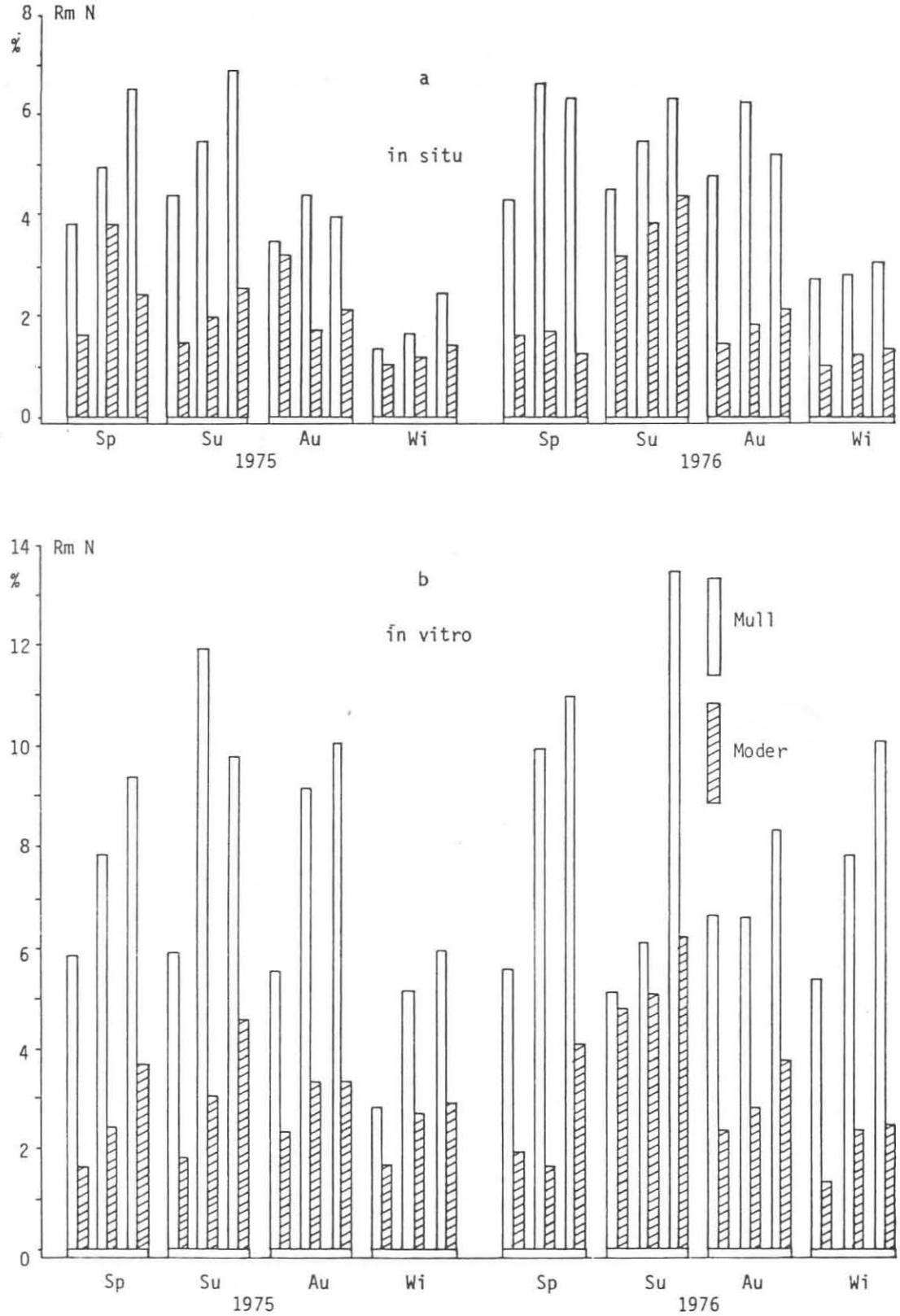

Fig. 4. Rate of mineralization of organic nitrogen (Rm, in %)
(a) *in situ* (b) *in vitro*

Fig. 2 shows the mineralization of soil organic nitrogen *in situ* at constant humidity (samples incubated in metallic boxes). In this case the microbial activity was subjected only to thermal fluctuation. The accumulation of mineral N was about two to three times higher in the anmoor than in the mull. In most cases the amount increased with the length of incubation. However, the rate of increase was not uniform. In all cases, excepting one(anmoor; spring, 1975), mineral N accumulation was higher during the first 3 weeks than in the following 6 and 9 weeks. In some cases a fall in values was encountered which suggested a possible biological immobilization (Billes *et al.*, 1971; Faille, 1975; Robertson & Tiedje, 1984) and/or denitrification (Broadbent & Clark, 1965; Robertson, 1964; Robertson & Tiedje, 1984; Rashid & Schaefer, 1986, 1987). In both soils, NO_3^- accumulation was much higher than that of NH_4^+. This indicates that the nitrifiers were abundant in both soils.

In the mull the accumulation of mineral N was relatively higher in the spring and summer and lower in the autumn and winter during both years. Similar results were obtained by Runge (1974) in an acid soil under coniferous vegetation. However, the very low value obtained in the 1976 winter might be due to comparatively low temperatures recorded during this season (Rashid 1977). In the anmoor the mineralization activity was relatively higher in the spring and summer and lower in the autumn and winter during 1975. But in 1976 comparatively higher values were obtained in the summer followed by gradually lowering of values in the autumn, spring and winter. The absolute winter minimum in both soils was undoubtedly linked with low winter temperature which reduced the activities of the mesophilic microbes. Slight variation in the highest values obtained in these soils during the two years of study might be due to sharp difference in climate. In 1976 the region suffered an unusual drought accompanied by relatively low winter/spring and high summer/autumn temperatures (Rashid, 1977). Microbial activities during incubation studies were observed to be influenced by the past pedoclimate (Billes *et al.*, 1974a, b; 1975; Rashid & Schaefer, 1985).

Fig. 3 shows the mineralization of soil organic nitrogen *in vitro* in different seasons during 1975 and 1976. The accumulation of mineral N in the anmoor was almost twice as high as in the mull in all cases. In the mull the accumulation of mineral N was more or less comparable in all seasons during 1975. However, the value obtained in the spring was somewhat higher. In 1976 it was at a maximum in the summer and at a minimum in the autumn, in the same soil. In the anmoor the maximum accumulation was observed in the summers and the minimum in the spring (1975) or in the winter (1976).

The circles inscribed on the curves (fig. 3) correspond to the accumulation of mineral N determined at the end of 21 d of incubation *in vitro*. In this case forced ventilation was applied to ensure aeration. The position of the circles on, or near to the curves reflects the degree of aeration of both soils and the reproducibility of the results.

In both soils the process of nitrification was so active that there was little ammonium left (fig. 3). A similar pattern was observed during the *in situ* study. This suggests that nitrification is ammonium-limited (Robertson, 1984). Moreover, the immobilization and/or dissimilatory reduction of NO_3^- appeared only episodically and briefly (in both cases of incubation), indicating that the liberation of easily assimilable carbon was feeble but regular. In these soils nitrogen mineralization activity was high and followed a seasonal rhythm. In tropical soils the optimum temperatures for ammonification and nitrification were found to be around 50 °C and 35 °C respectively (Myers, 1975). However, in temperate regions other factors such as aeration, *p*H and availability of substrates essentially govern the nitrification processes (Dommergues & Mangenot, 1970).

In acid mull (*p*H 4.5) Rashid & Schaefer (1984) could not isolate *in vitro* the autotrophic ammonia and nitrite oxidizing bacteria. In this soil the fungi dominated the microflora. Heterotrophic nitrification, carried out by a number of fungi and bacteria has been reported (Schmidt, 1954; Eylar & Schmidt, 1959; Doxtader & Alexander, 1966; Ishaque & Cornfield, 1972, 1976; Alexander, 1977; Focht & Verstraete, 1977; Choudhury & Cornfield, 1978). In the mull, at least partial heterotrophic nitrification might not be ruled out. The formation of NO_3^- in highly acid soils was also reported by others (Weber & Gainey,

1962; VAN PRAAG & WEISSEN, 1972; CHOUDHURY & CORNFIELD, 1978; KLEIN *et al.*, 1983). However, no NO_3^- formation (SAHRAWAT, 1980; WHEELER & DONALDSON, 1983) or low capacities for nitrification (THEOBALD & SMITH, 1974) in acid soils are a common feature. Whereas in the anmoor, autotrophic nitrification is well established (RASHID & SCHAEFER, 1984).

The rate of mineralization (Rm) of organic N *in situ* in the mull was maximum in the summer and spring and minimum in the winter in 1975 (fig. 4a). In 1976 the Rm was relatively higher and comparable to the values in the spring, summer and autumn and feeble in the winter (fig. 4a). In the anmoor it was slightly higher in the spring, followed by decreasing values in the autumn, summer and winter in 1975. In 1976 the Rm was maximum in the summer followed by decreasing values in the autumn, spring and winter. The lowest winter values in all cases might be partially due to low microbial activities and an incrase in the total-N content of soil as a result of the incorporation of litters during the preceeding fall.

The Rm *in vitro* increased with the duration of incubation in most cases with the exception of the summer of 1975 and spring of 1976 where a fall in value was recorded in the acid mull and anmoor respectively (fig. 4b). This irregularity might be due to microbial immobilization and/or dissimilatory reduction of NO_3^-. In both soils the Rm highest in the summers (fig. 4b) and lowest in the winters or autumn (mull: 1975). The rate of mineralization was higher in these soils than that obtained by LEMÉE (1967) and much higher than that suggested by DUCHAUFOUR (1977) for forest mulls. Even higher values were obtained by BILLES *et al.* (1975) under a Mediterranean ecosystem.

The apparent anomaly observed between the accumulation of mineral N (much higher in anmoor than in mull) and the rate of mineralization (very high in mull as compared to anmoor) under both conditions of incubation was due to the fast that the anmoor, a hydromorphic soil, situated at the base of the catena, presented a condition where anaerobic processes of decomposition of organic materials led to the accumulation of intermediate products (ALEXANDER, 1977). Whereas in the mull, a well aerated soil, the accumulation of such products could not be expected. In the anmoor, the processes of humification were preponderant over those of mineralization causing an increase in the total-N content. The reverse was true in the case of the mull and hence this humus was biologically more active than that in the anmoor. RASHID & SCHAEFER (1985) observed that the rate of mineralization of carbon was also higher in mull than in anmoor, in the same soil samples.

4. References

ALEXANDER, M., 1977. Introduction to soil microbiology. 2nd Ed., Wiley, New York, 465 p.

BILLES, G., J. CORTEZ & P. LOSSAINT, 1971 a. L'activité biologique des sols dans les écosystèmes méditerranéens. I. Minéralisation du carbone. Rev. Écol. Biol. Sol **8**, 375—395.

— P. LOSSAINT & J. CORTEZ, 1971 b. L'activité biologique des sols dans les écosystèmes méditerranéens. II. Minéralisation de l'azote. Rev. Écol. Biol. Sol **8**, 533—552.

— J. CORTEZ & P. LOSSAINT, 1975. Étude comparative de l'activité biologique des sols sous peuplements arbustifs et herbacés de la garrigue méditerranéenne. I. Minéralisation du carbone et de l'azote. Rev. Écol. Biol. Sol **12**, 115—139.

BORMAN, F. H., G. E. LIKENS & J. M. MELILLO, 1977. Nitrogen budget for an aggrading northern hardwood forest ecosystem. Science **196**, 981—983.

BREMNER, J. M., 1965. Methods of soil analysis (Ed. C. A. Black et al.). Ser. Agron. No 9, published by Am. Soc. Agron. Inc., Wisc. USA. Vol. 2, 1330—1341.

BROADBENT, F. E., & F. E. CLARKE, 1965. Denitrification. *In:* Soil nitrogen (Bartholomew, W. V , & F. E. Clark, Eds.). Am. Soc. Agron. Madison, Wisconsin, pp. 344—359.

CAMPBELL, C. A., R. J. K. MYERS & K. L. WEIER, 1981. Nitrogen mineralization potentials, decomposition rates and their relationship to temperature for five Queensland soils. Aust. J. Soil Res. **19**, 323—332.

CHOUDHURY, M. S., & A. H. CORNFIELD, 1978. Nitrogen and carbon mineralization during incubation of two Bangladesh soils in relation to temperature. Plant and Soil **49**, 317—321.

DOMMERGUES, Y., & F. MANGENOT, 1970. Écologie microbienne du sol. Masson, Paris, 769 pp.

DOXTADER, K. G., & M. ALEXANDER, 1966. Nitrification by heterotrophic soil microorganisms. Soil Sci. Soc. Am. Proc. **30**, 351—355.

Duchaufour, Ph., 1977. Pédologie. Masson, Paris, 477 pp.
Eylar, O. R. Jr., & E. L. Schmidt, 1959. A survey of heterotrophic microorganisms from soil for ability to form nitrite and nitrate. J. Gen. Microbiol. **20**, 473—481.
Faille, A., 1975. Recherches sur les écosystèmes des réserves biologiques de la forêt de Fontainebleau. V. Evolution à court terme des humus à la suite de l'ouverture de clairières. Oecol. Plant. **10**, 43—62.
Focht, D. D., & W. Verstraete, 1977. Biochemical ecology of nitrification and denitrification. In: Alexander, M. (ed.), Advances in microbial ecology. **1**, Plenum Press, New York, 135—124 pp.
Freney, J. R., O. T. Denmead & J. R. Simpson, 1978. Soil as a source or sink for atmospheric nitrous oxide. Nature, London **273**, 530—532.
Herlihy, M., 1979. Nitrogen mineralization in soils of varying texture, moisture and organic matter. I. Potential and experimental values in fallow soils. Plant and Soil **53**, 255—267.
Ishaque, M., & A. H. Cornfield, 1972. Nitrogen mineralization and nitrification during incubation of East Pakistan "tea" soils in relation to *p*H. Plant and Soil **37**, 91—95.
— — 1976. Evidence for heterotrophic nitrification in an acid Bangladesh soil lacking autotrophic nitrifying microorganisms. Trop. Agric. (Trinidad) **53**, 157—160.
Juma, N. G., E. A. Paul & B. Mary, 1984. Kinetic analysis of net nitrogen mineralization in soil. Soil Sci. Soc. Am. J. **48**, 753—757.
Keeney, D. R., 1980. Prediction of soil nitrogen availability in forest ecosystems: a literature review. For. Sci. **26**, 159—171.
Lemée, G., 1967. Investigations sur la minéralisation de l'azote et son évolution annuelle dans des humus forestiers *in situ*. Oecol. Plant **2**, 285—324.
MacRae, I. C., R. R. Ancajas & S. Salandanan, 1968. The fate of nitrate nitrogen in some tropical soils following submergence. Soil Sci. **105**, 327—334.
Myers, J. K. R., 1975. Temperature effects on ammonification and nitrification in a tropical soil. Soil Biol. Biochem. **7**, 79—82.
Piper, C. S., 1960. Soil and plant analysis. Adelaide Univ. Press. 368 pp.
Rashid, G. H., 1977. Dynamique de la minéralisation du carbone et de l'azote dans deux sols d'une «catena» forestière: Incidence de l'hydromorphie. Thèse Docteur-Ingénieur, Univ. Paris-XI, 123 pp.
— & R. Schaefer, 1984. Cellulolytic activity in two temperate forest catena soils (Orsay). Rev. Ecol. Biol. Sol **21**, 431—438.
— — 1985. The seasonal pattern of carbon dioxide evolution from two temperate forest catena soils. Rev. Biol. Sol **22**, 419—431.
— — 1986. Denitrification studies in a temperate forest catena soil. I. Formation of ammonium during nitrate reduction. Plant and Soil **93**, 367—372.
— — 1987. Seasonal rate of nitrate reduction in two temperate forest soils. Plant and Soil **97**, 291 to 294.
Robertson, G. P., 1984. Denitrification and nitrogen mineralization in a lowland rainforest succession in Costa Rica, Central America. Oecologia (Berlin) **61**, 99—104.
— & J. M. Tiedje, 1984. Denitrification and nitrous oxide production in successional and old-growth Michigan forests. Soil Sci. Soc. Am. J. **48**, 383—389.
Runge, M., 1974. Die Stickstoff-Mineralisation im Boden eines Sauerhumusbuchenwaldes. I. Mineralstickstoff-Gehalt und Netto-Mineralisation. Oecol. Plant. **9**, 201—218.
Sahrawat, K. L., 1980. Nitrogen mineralisation in acid sulfate soils. Plant and Soil **57**, 143—146.
Schmidt, J. E. L., 1954. Nitrate formation by a soil fungus. Science **119**, 187—189.
Sollins, P., G. Spycher & C. A. Glassman, 1984. Net nitrogen mineralization fom light- and heavy-fraction forest soil organic matter. Soil. Biol. Biochem. **16**, 31—57.
Stanford, G., & E. Epstein, 1974. Nitrogen mineralization-water relations in soils. Soil Sci. Soc. Am. Proc. **38**, 103—107.
Theobald, W. F., & W. H. Smith, 1974. Nitrate production in two forest soils and nitrate reduction in pine. Soil Sci. Soc. Am. Proc. **38**, 668—672.
van Praag, H. J., & F. Weissen, 1973. Elements of a functional definition of oligotroph humus based on the nitrogen nutrition of forest stands. J. Appl. Ecol. **10**, 569—583.
Vitousek, P. M., J. R. Gosz, C. C. Grier, J. M. Melillo, W. A. Reiners & R. L. Todd, 1979. Nitrate loss from disturbed ecosystems. Science **204**, 469—474.
Weber, D. F., & P. L. Gainey, 1962. Relative sensitivity of nitrifying organisms to hydrogen ions in soils and solutions. Soil Sci. **94**, 138—145.
Wheeler, G. L., & J. M. Donaldson, 1983. Nitrification in an upland forest sere. Soil Biol. Biochem **15**, 119—121.

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The changes in mineral N content *in situ* and in net nitrogen mineralization during incubation studies were investigated along two yearly cycles in the humiferous horizon of two temperate forest catena soils.

The mineral N content varied in a irregular pattern from one year to another in both soils. However, an absolute minimum in the winter in all cases indicated low microbial activities and possible leaching out of NO_3^- during the season.

The net N-mineralization during incubation *in situ* showed a seasonal evolution which was principally temperature-dependent. As a result, the mineralization activities and the rate of mineralization were lowest in the winter and highest in the spring or in the summer depending on the climate.

In the mull the net N-mineralization *in vitro* was more or less uniform in different seasons during 1975 but during 1976 it was highest in the summer and lowest in the autumn in the same soil. In the anmoor, this value was maximum in the summer and minimum either in the spring (1975) or in the winter (1976). The rate of mineralization *in vitro* was highest in the summer in both soils and lowest either in the winter or in the autumn.

The mineral N content and net N-mineralization during incubation studies were higher in the anmoor whilst the rate of mineralization was much higher in the mull, indicating thereby higher microbial activities in the latter soil. Under natural conditions NH_4^+ accounted for a larger share of mineral N whereas during incubation studies NO_3^- represented early all of the total mineral N.

Key words: Anmoor, Mull, Seasonal dynamics, Soil nitrogen mineralization, Temperate forest catena.